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Substitute Specification

**HYDRAZINE-BASED AND CARBONYL-BASED BIFUNCTIONAL
CROSSLINKING REAGENTS****RELATED APPLICATIONS**

This application is a divisional of U.S. patent 6,800,728, patent application No.: 09/815,978, filed March 22, 2001 entitled "HYDRAZINE-BASED AND CARBONYL-BASED BIFUNCTIONAL CROSSLINKING REAGENTS" which claims priority to U.S. provisional patent application No. 60/191,186, filed March 22, 2000, to Schwartz, entitled "NOVEL

- 10 HYDRAZINE-BASED AND CARBONYL-BASED BIFUNCTIONAL CROSSLINKING REAGENTS." The disclosures of the above-referenced applications are incorporated herein in their entirety.

FIELD OF THE INVENTION

- 15 The present disclosure may be applied in general to the field of chemistry, more particularly in the area of crosslinking reagents.

BACKGROUND OF THE INVENTION

- Methods to crosslink biomolecules such as proteins, oligonucleotides and carbohydrates to each other, to radioactive and non-radioactive metal chelates, to drugs and to surfaces have allowed development of both in
20 vitro and in vivo diagnostic assays as well as in vivo therapies. A wide variety of methods have been developed and reviewed (Greg T. Hermanson, Bioconjugate Techniques, Academic Press).

- There are a limited number of crosslinking couples, i.e., maleimide/thiol and bromoacetamide/thiol, that are routinely used to
25 prepare conjugates for diagnostic and therapeutic uses. These reagents have limitations in that at high protein concentrations (i.e., >5 mg/mL) protein/protein crosslinking may occur. Also, the maleimido-modified moieties have a limited half-life due to hydrolysis at neutral and basic pH. Incorporation of thiol moieties on biomolecules requires both a coupling
30 and a subsequent activation step. The resultant thiol-modified proteins can readily oxidize to form disulfide polymerized proteins. Also macromolecules containing disulfide bonds, i.e., antibodies, are readily